# The Sequence of a 1.8-Mb Bacterial Linear Plasmid Reveals a Rich Evolutionary Reservoir of Secondary Metabolic Pathways

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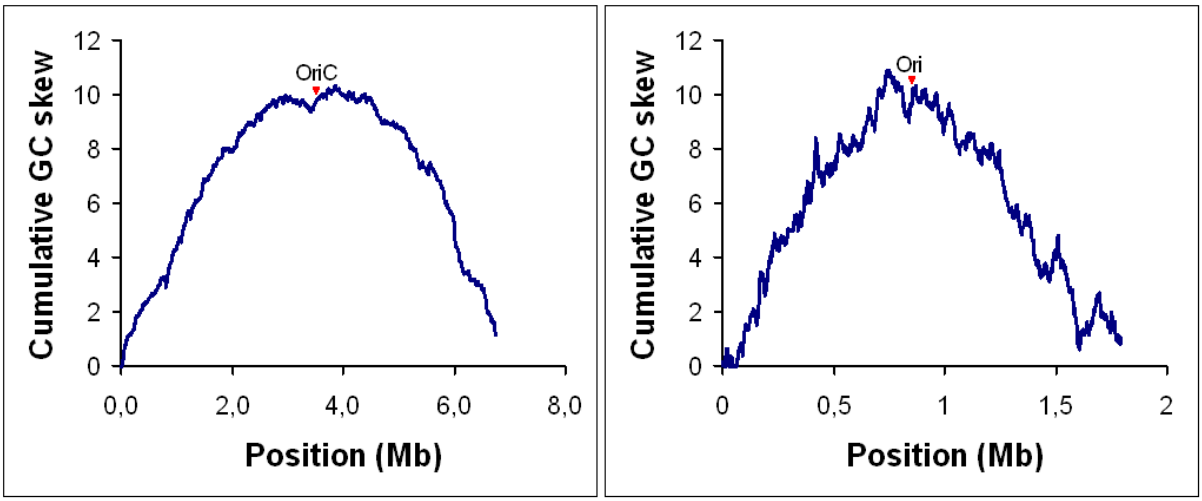
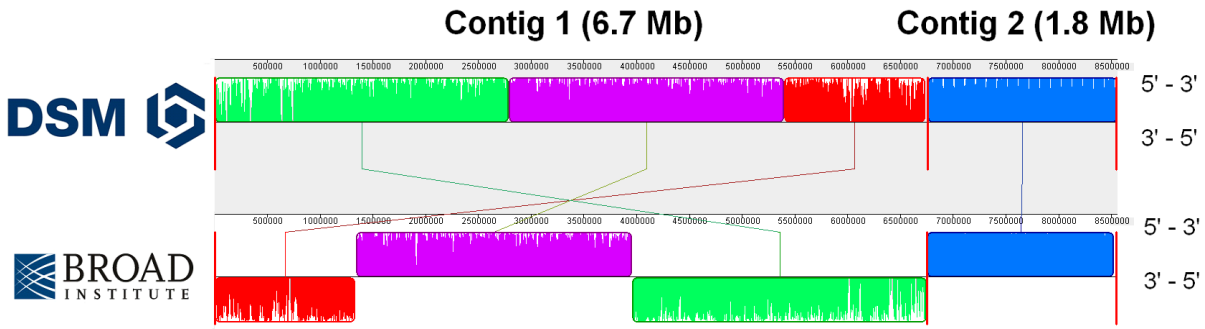


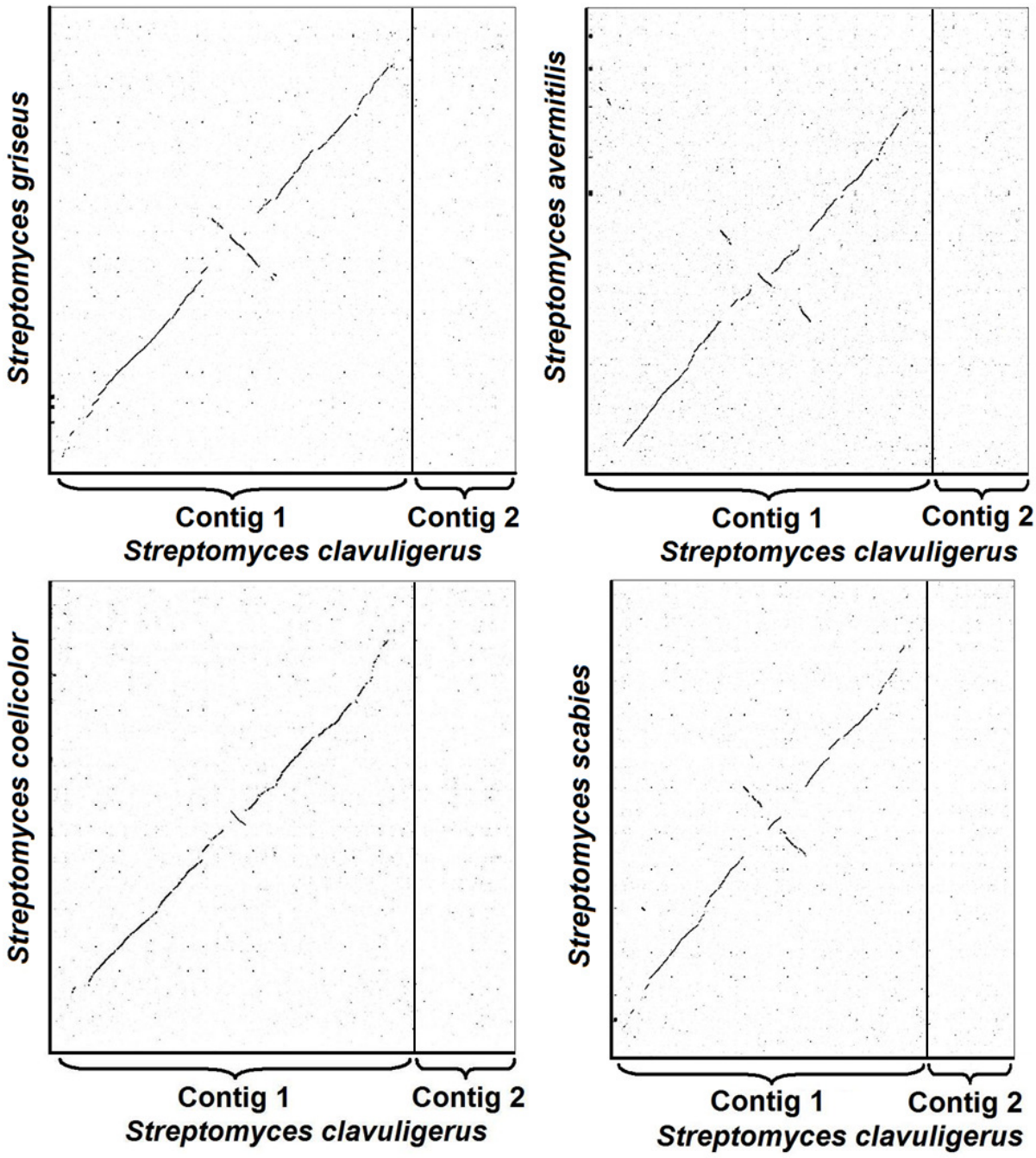

**Supplementary Figure S1**

Cumulative GC skew diagrams of the two contigs identified in the *S. clavuligerus* genome assembly. The position of the predicted origins of replication are shown as a red triangle. GC skew was calculated using GenSkew (http://mips.gsf.de/services/analysis/genskew/).



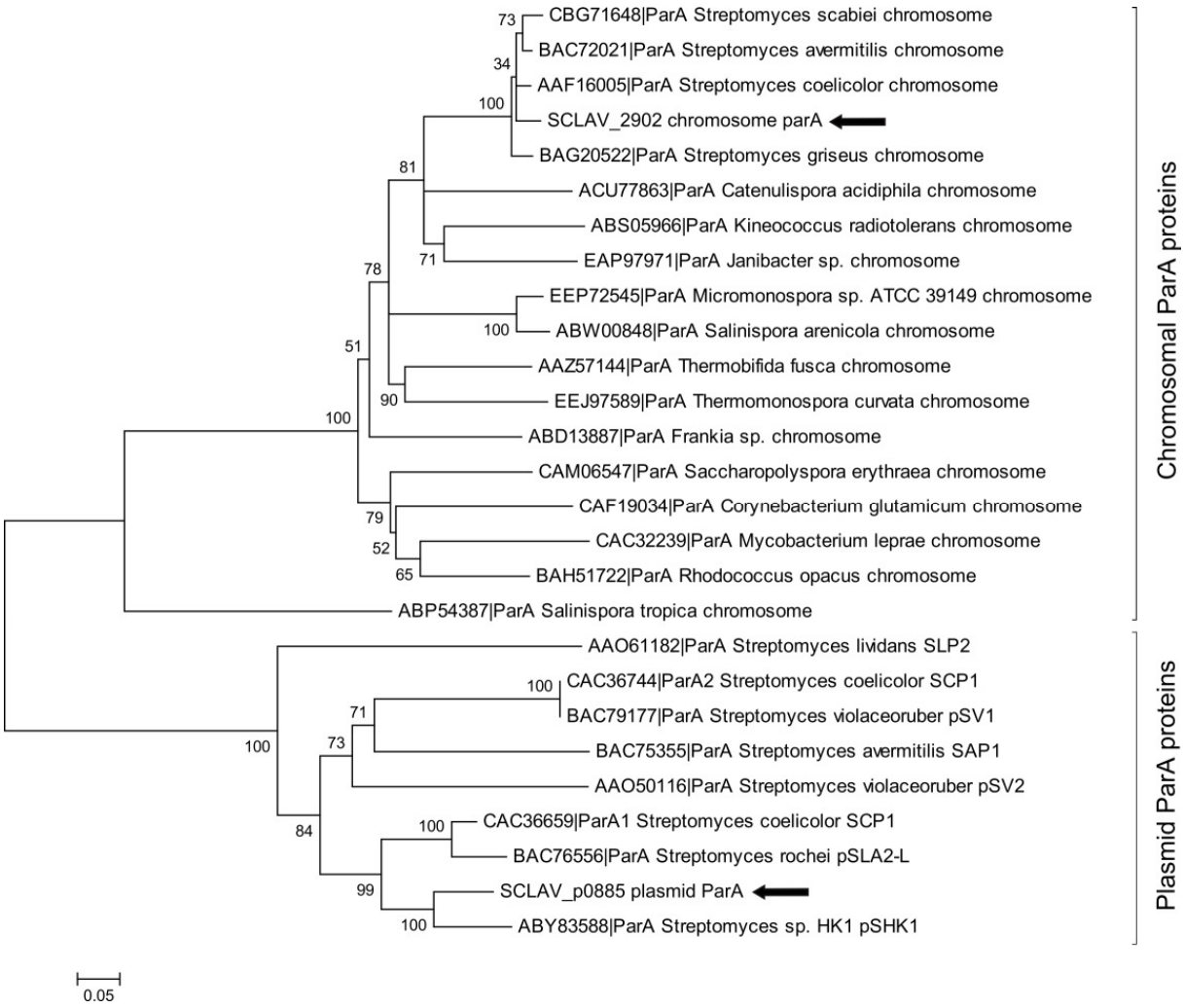

**Supplementary Figure S2**

Mauve (Darling et al. 2004) genome alignment of the DSM/JCVI and Broad Institute *S. clavuligerus* draft genome sequences. A large inversion of over 2.5 Mb is visible on the chromosome.



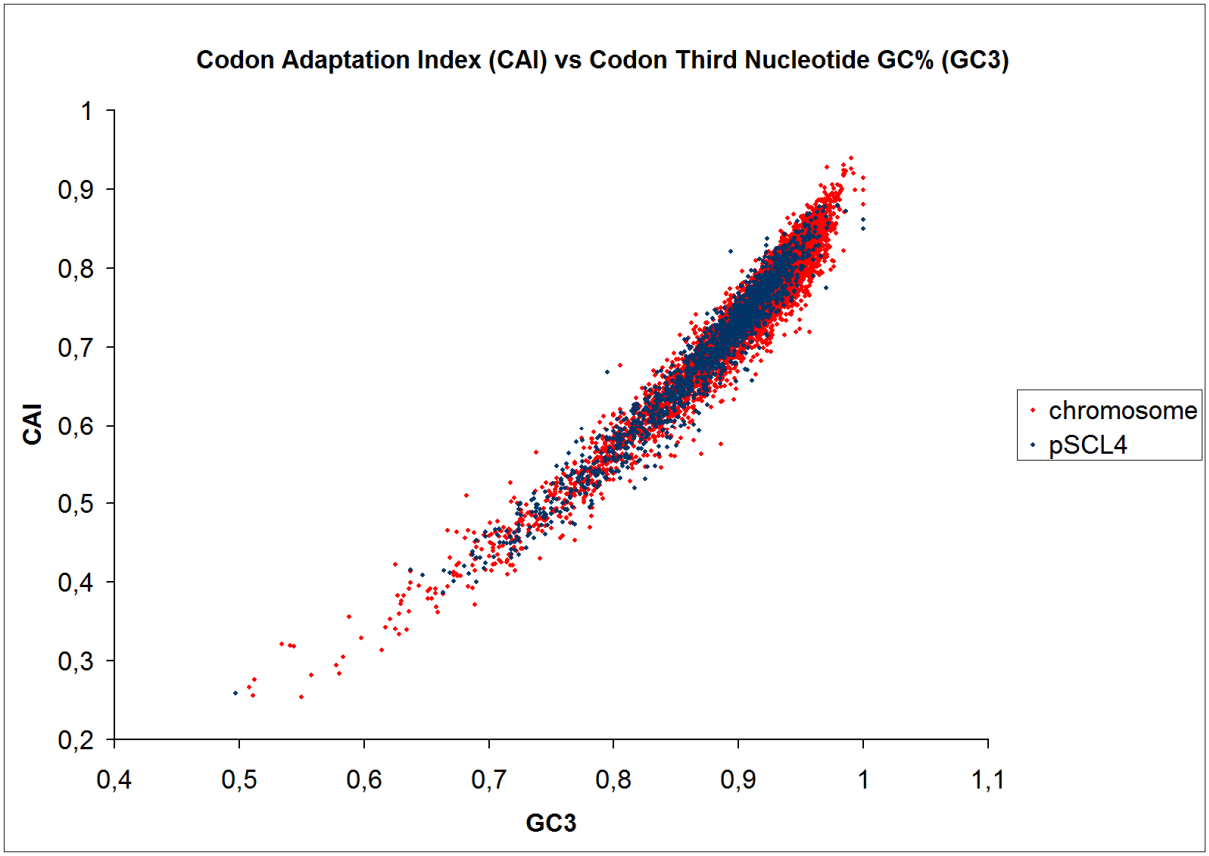

**Supplementary Figure S3**

Dot-plot comparisons of the *S. clavuligerus* genome with the four *Streptomyces* genomes completed so far. The dot-plots were constructed using Gepard (Krumsiek et al. 2007) on the complete nucleotide sequences of the composite genome of *S. clavuligerus*, and of the chromosomes of *S. coelicolor* A3(2), *S. griseus* IFO 13350, *S. avermitilis* ATCC 31267 and *S. scabies* 87.22.



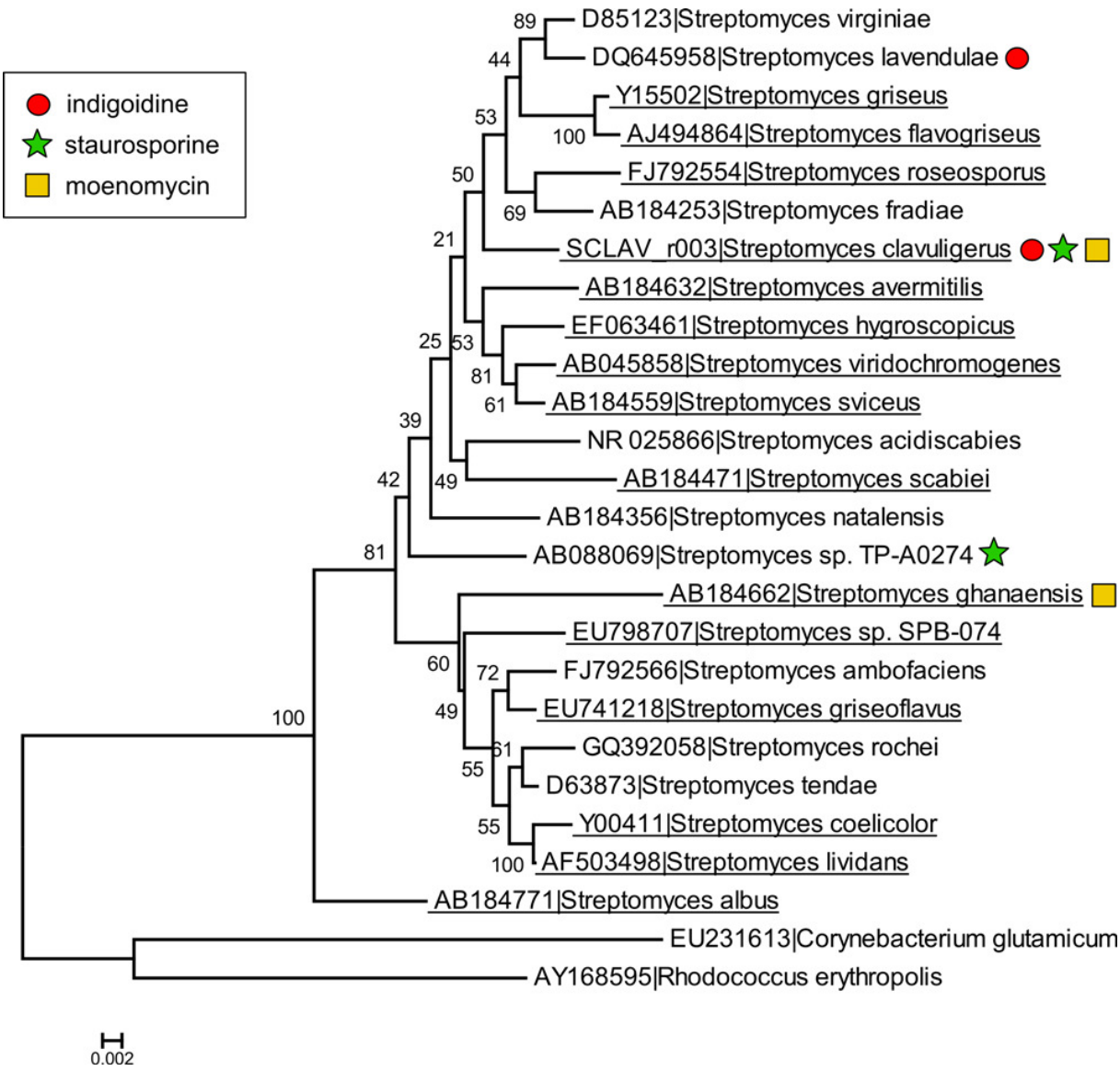

**Supplementary Figure S4**

Phylogeny of ParA proteins, which shows the distinctiveness of the plasmid and chromosomal maintenance machineries. Phylogenies were calculated using the NJ method in MEGA 4 (Tamura et al. 2007). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) are shown next to the branches.




**Supplementary Figure S5**

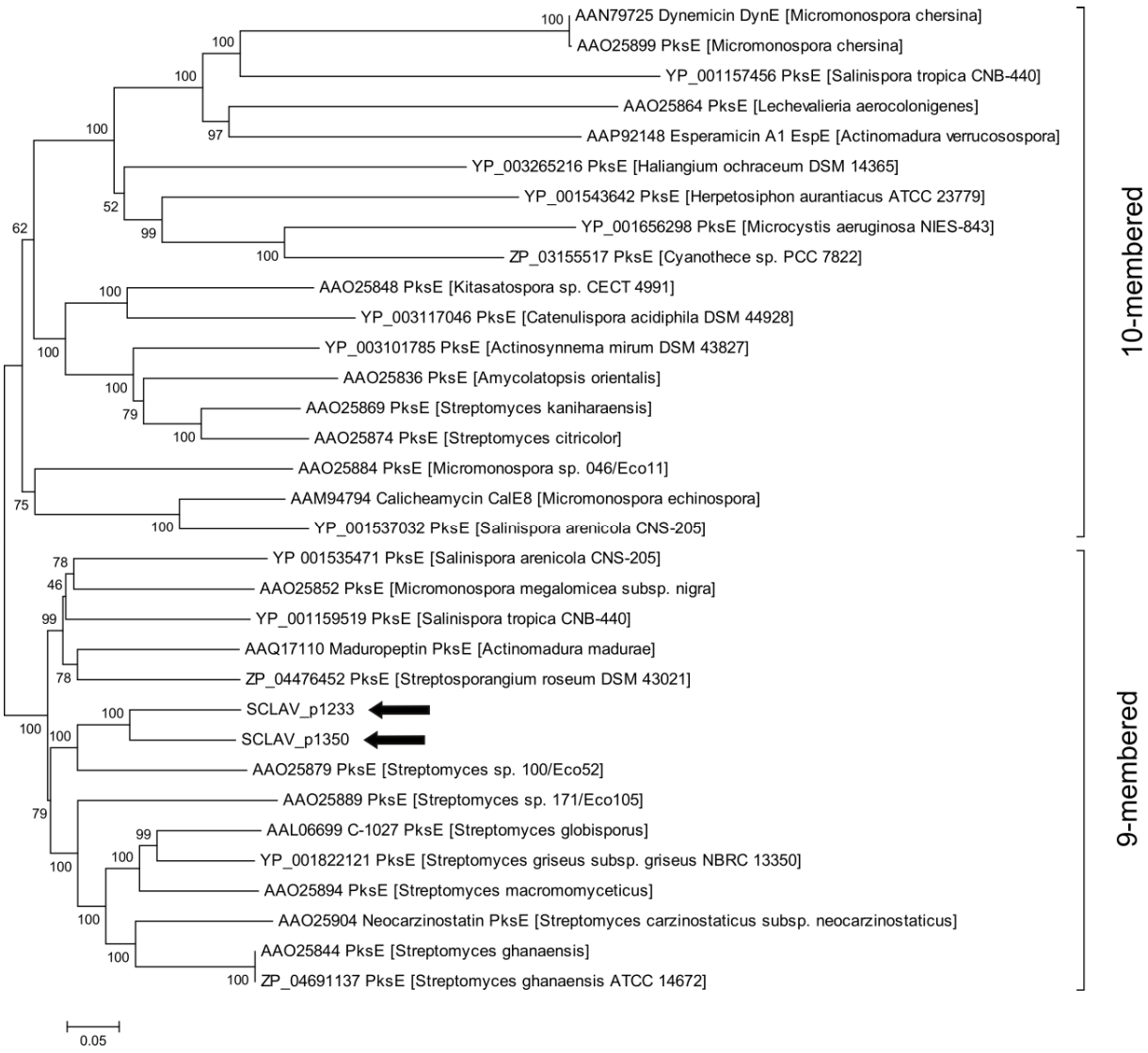
Distribution of all *S. clavuligerus* proteins from the chromosome and plasmid as to their Codon Adaptation Index (CAI) and third codon position GC % (GC3). CAI and GC3 were calculated with CAIcal (Puigbo et al. 2008) and CodonW (http:// codonw.sourceforge.net).




**Supplementary Figure S6**

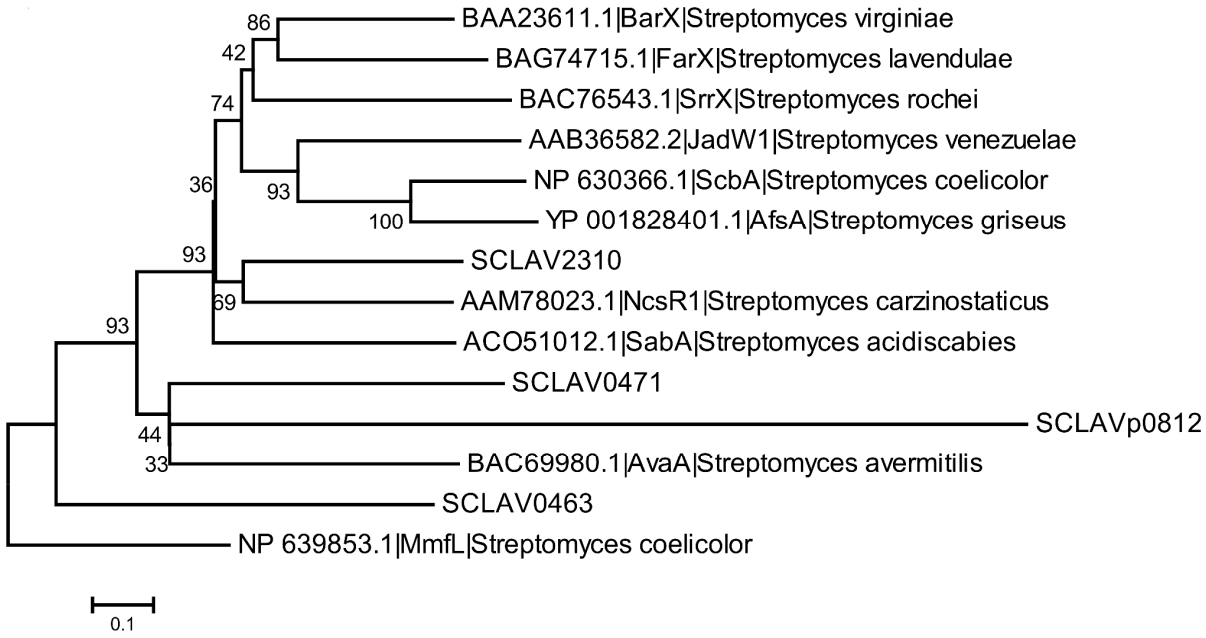
16S ribosomal RNA-based phylogenetic tree of *Streptomyces* species, showing the distribution of known moenomycin (yellow rectangle), staurosporine (green star) and indigoidine (red circle) gene clusters. The names of species of which either a complete genome or an almost complete draft genome is available are underlined. The dispersed absence/presence pattern indicates a probable role of horizontal gene transfer in the evolutionary history of these gene clusters. Phylogenies were calculated using the minimum evolution method in MEGA 4 (Tamura et al. 2007). The percentage of replicate trees in which

the associated taxa clustered together in the bootstrap test (100 replicates) are shown next to the branches.




**Supplementary Figure S7**

Phylogeny of enediyne-type PKSs, showing the position of the two enediyne-type PKSs detected on the megaplasmid pSCL4. Phylogenies were calculated using the neighbour-joining method in MEGA 4 (Tamura et al. 2007). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) are shown next to the branches.




**Supplementary Figure S8**

Phylogeny of AfsA-domain containing proteins, showing the clustering of SCLAV_2310 with the known γ-butyrolactone biosynthesis proteins ScbA and AfsA and their known homologs BarX, FarX, SrrX, JafW1, NcsR1 and SabA. The Mm furan biosynthesis protein MmfL from *S. coelicolor* (Corre et al. 2008) was used as an outgroup. Phylogenies were calculated using the neighbour-joining method in MEGA 4 (Tamura et al. 2007). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) are shown next to the branches.

| Query (from *S. griseus*) | Hit | Identity (%) | Annotation | Unique to plasmid? |
|---|---|---|---|---|
| BAG20055.1\|putative_cold_shock_protein | SCLAV_p0115 | 62.9 | Cold-shock domain family protein | no |
| BAG20062.1\|putative_ABC-transporter_ATP-binding_protein | SCLAV_p0477 | 71.29 | ABC transporter ATP-binding protein | yes |
| BAG19908.1\|putative_transcriptional_regulator | SCLAV_p0530 | 69.04 | Transcriptional regulator | yes |
| BAG19876.1\|putative_TetR-family_transcriptional_regulator | SCLAV_p0668 | 63.04 | Putative TetR-family transcriptional regulator | yes |
| BAG20858.1\|putative_ABC_transporter_ATP-binding_protein | SCLAV_p0830 | 60.79 | Daunorubicin resistance ABC transporter ATP-binding subunit | yes |
| BAG20505.1\|conserved_hypothetical_protein | SCLAV_p0866 | 66.76 | Methicillin resistance protein | no |
| BAG21602.1\|putative_UvrA-like_protein | SCLAV_p0905 | 80.7 | UvrABC system protein A | no |
| BAG18126.1\|putative_malate_synthase | SCLAV_p0928 | 61.44 | Malate synthase | no |
| BAG18044.1\|putative_NAD-dependent_aldehyde_dehydrogenase | SCLAV_p0939 | 86.17 | Methylmalonate-semialdehyde dehydrogenase | yes |
| BAG18460.1\|putative_monooxygenase | SCLAV_p0942 | 66.67 | 2-polyprenyl-6-methoxyphenol hydroxylase-like oxidoreductase | yes |
| BAG17193.1\|putative_methyltransferase | SCLAV_p1166 | 74.27 | Putative methyltransferase | yes |
| BAG19507.1\|putative_cation_transport_system_component | SCLAV_p1322 | 62.13 | Potassium-transporting ATPase subunit B | no |
| BAG20340.1\|putative_ATP-dependent_RNA_helicase | SCLAV_p1485 | 62.79 | ATP-dependent RNA helicase | no |
| BAG22539.1\|putative_iron-siderophore_uptake_ABC_transporter_ATP-binding_protein | SCLAV_p1592 | 67.33 | Ferric enterobactin transport ATP-binding protein FepC | no |

## Supplementary Table S1

Core genome hits on the 1.8 Mb replicon.

| Name | Genes | Type | Putative function / remarks |
|---|---|---|---|
| SMCp1 | SCLAV_p0064 – SCLAV_p0075 | Terpene synthase | |
| SMCp2 | SCLAV_p0286 – SCLAV_p0308 | NRPS & PKS-NRPS | |
| SMCp3 | SCLAV_p0326 – SCLAV_p0334 | Terpene synthase & Type II PKS | Pentalenene synthase |
| SMCp4 | SCLAV_p0486 – SCLAV_p0492 | Terpene synthase | |
| SMCp5 | SCLAV_p0509 – SCLAV_p0520 | Type II PKS | |
| SMCp6 | SCLAV_p0563 – SCLAV_p0588 | NRPS / PKS / Terpene synthase | TS = Pentalenene synthase |
| SMCp7 | SCLAV_p0633 – SCLAV_p0643 | Terpene synthase | Germacradienol/germacrene D synthase |
| SMCp8 | SCLAV_p0690 – SCLAV_p0714 | Other | |
| SMCp9 | SCLAV_p0760 – SCLAV_p0768 | Terpene synthase | Pentalenene synthase |
| SMCp10 | SCLAV_p0966 – SCLAV_p0986 | Terpene synthase | Pentalenene synthase, terpene cyclase |
| SMCp11 | SCLAV_p1005 – SCLAV_p1012 | PKS-NRPS | |
| SMCp12 | SCLAV_p1057 – SCLAV_p1063 | Actin-ADP-ribosylating toxin | |
| SMCp13 | SCLAV_p1074 – SCLAV_p1079 | Beta-lactam | 'Paralogous' alanylclavam cluster |
| SMCp14 | SCLAV_p1107 – SCLAV_p1123 | Indolocarbazole | Staurosporine gene cluster |
| SMCp15 | SCLAV_p1170 – SCLAV_p1194 | Terpene synthase | Pentalenene synthase (2x) |
| SMCp16 | SCLAV_p1219 – SCLAV_p1234 | Enediyine PKS | |
| SMCp17 | SCLAV_p1263 – SCLAV_p1273 | PKS-NRPS | |
| SMCp18 | SCLAV_p1274 – SCLAV_p1290 | Phosphoglycolipid | Moenomycin gene cluster |
| SMCp19 | SCLAV_p1302 – SCLAV_p1311 | NRPS | |
| SMCp20 | SCLAV_p1334 – SCLAV_p1341 | NRPS | NRPS fused to major facilitator transporter in final module |
| SMCp21 | SCLAV_p1345 – SCLAV_p1376 | Enediyne PKS | |
| SMCp22 | SCLAV_p1407 – SCLAV_p1415 | Terpene synthase | Pentalenene synthase |
| SMCp23 | SCLAV_p1426 – SCLAV_p1432 | NRPS / Terpene synthase | |
| SMCp24 | SCLAV_p1471 – SCLAV_p1483 | NRPS | Indigoidine synthetase |
| SMCp25 | SCLAV_p1508 – SCLAV_p1510 | Possible beta-lactam cluster | |
| | | | |
| SMC1 | SCLAV_0001 – SCLAV_0026 | Macrolide type I PKS | |
| SMC2 | SCLAV_0082 – SCLAV_0105 | Other | Putative secondary metabolite biosynthesis cluster without known homologs |
| SMC3 | SCLAV_0148 – SCLAV_0152 | Siderophore | |
| SMC4 | SCLAV_0153 – SCLAV_0172 | Terpene synthase | Germacradienol synthase-like |
| SMC5 | SCLAV_0446 – SCLAV_0497 | Type I PKS | |
| SMC6 | SCLAV_1942 – SCLAV_1955 | Siderophore | Desferrioxamines cluster |
| SMC7 | SCLAV_2274 – SCLAV_2302 | NRPS | |
| SMC8 | SCLAV_2456 – SCLAV_2469 | Lantibiotic | |
| SMC9 | SCLAV_2920 – SCLAV_2935 | Beta-lactam | Clavams |
| SMC10 | SCLAV_4178 – SCLAV_4197 | Beta-lactam | Clavulanic Acid |
| SMC11 | SCLAV_4198 – SCLAV_4217 | NRPS / Beta-lactam | Cefamycin C |
| SMC12 | SCLAV_4387 – SCLAV_4392 | Lantibiotic | |
| SMC13 | SCLAV_4460 – SCLAV_4486 | PKS-NRPS | |
| SMC14 | SCLAV_4740 – SCLAV_4758 | NRPS | NRPS-Beta-lactamase fusion in one module |
| SMC15 | SCLAV_4911 – SCLAV_4921 | Type II PKS | Putative spore pigment (WhiEI and WhiEII homologs) |
| SMC16 | SCLAV_5142 – SCLAV_5148 | NRPS | |
| SMC17 | SCLAV_5227 – SCLAV_5255 | Phytoene / squalene | |
| SMC18 | SCLAV_5267 – SCLAV_5278 | NRPS | |
| SMC19 | SCLAV_5325 – SCLAV_5347 | NRPS | |
| SMC20 | SCLAV_5463 – SCLAV_5497 | Type III PKS | |
| SMC21 | SCLAV_5608 – SCLAV_5638 | PKS-NRPS | |
| SMC22 | SCLAV_5670 – SCLAV_5674 | Terpene synthase | |
| SMC23 | SCLAV_5702 – SCLAV_5710 | Siderophore | IucA/IucC |

**Supplementary Table S2**

Secondary metabolism gene clusters in the S. clavuligerus genome. SMC = secondary metabolite cluster. SMC1-23 are the chromosome SMCs, SMCp1-p25 are the plasmid SMCs.

| Gene | Annotation |
|---|---|
| SCLAV_0170 | Beta-lactamase |
| SCLAV_1453 | Alkaline D-peptidase |
| SCLAV_1622 | Beta-lactamase |
| SCLAV_1657 | Beta-lactamase |
| SCLAV_2072 | D-alanyl-D-alanine carboxypeptidase |
| SCLAV_2296 | Putative esterase |
| SCLAV_2436 | Beta-lactamase |
| SCLAV_2579 | Beta-lactamase |
| SCLAV_2874 | Beta-lactamase |
| SCLAV_3000 | D-alanyl-D-alanine carboxypeptidase |
| SCLAV_3128 | Alkaline D-peptidase |
| SCLAV_3176 | Alkaline D-peptidase |
| SCLAV_3177 | Alkaline D-peptidase |
| SCLAV_4216 | Beta-lactamase |
| SCLAV_4375 | Beta-lactamase |
| SCLAV_4749 | Non-ribosomal peptide synthetase with beta-lactamase domain |
| SCLAV_4822 | Alkaline D-peptidase |
| SCLAV_p0031 | Serine-type D-Ala-D-Ala carboxypeptidase |
| SCLAV_p0119 | D-stereospecific peptide hydrolase precursor |
| SCLAV_p0171 | Putative beta-lactamase |
| SCLAV_p0883 | Penicillin-binding protein, beta-lactamase class C |
| SCLAV_p1311 | Penicillin-binding protein, beta-lactamase class C |

## Supplementary Table S3

Beta-lactamase domain-containing proteins in the *S. clavuligerus* genome.

## References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